

Effect of chabazitic-zeolites and effective microorganisms on growth and chemical composition of *Aloe barbadensis* Miller and *Aloe arborescens* Miller

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With the aim of improving the growth of plants of *Aloe barbadensis* and *A. arborescens* and improve the content of sugars and minerals, several experiments were conducted replacing chabazitic-zeolites with normal inorganic substrates and adding the effective microorganisms (EM) to assess whether they affected plant development. The experiment on both *Aloe* species involved three treatments: 1) soil; 2) soil with addition of chabazitic-zeolites; and 3) soil with addition of chabazitic-zeolites and treated with EM. The results showed that the use of zeolites and EM microorganisms increased the quality characteristics of *Aloe* plants under cultivation; specifically plant growth, root development and production of metabolites useful for nutraceutics.

Key-words: plant quality, ornamental, symbiotic bacteria, alternative substrates, metabolites.

INTRODUCTION

The *Aloe barbadensis* Miller is a perennial plant that grows in the shape of a head, whose base is surrounded by a rosette of fat and thorny leaves with spiral-shaped evolution. Its structure and consistency are vaguely reminiscent of cactus. Originally from Africa, *Aloe barbadensis*, has spread across the Americas, after the expeditions of Columbus and Vespucci. *Aloe barbadensis* has fleshy, succulent, spotted green leaves with delicate contours, sometimes with pink dots during cold periods (Rodriguez-Garcia et al., 2007). As time passes, the bright green color tends to fade into grey green (Lawless and Allan, 2000). After 1950, plantations arose in the central-southern USA, specifically in Texas, Arizona and Florida. There are also some extensions in Mexico and in South America (Bassetti and Sala, 2001). *Aloe*

barbadensis Miller is currently the most widely used and well-known type of *Aloe* in the world. This is mainly attributable to the high yield of its leaves, its robustness and easy transformation into pulp to drink or gel for outdoor use (Anez and Vasquez, 2005).

Another species of *Aloe* is the *A. arborescens* Miller, which is native, like the *barbadensis*, to central-southern Africa. It is widespread in South Africa, and Asia, especially in Russia and Japan. The *Aloe arborescens*, unlike *A. vera*, does not develop on a single stump but extends on a central woody trunk, with alternating leaf growth, which can reach, when mature, a height of two or three meters. It is a perennial plant with fat, thorny leaves with spiral-shaped evolution, of green-grey color and less fleshy, filiform, of length between 50 and 60 cm and

weight from 10 to 100 g each (Bassetti and Sala, 2001).

Its narrow, filiform leaves have a larger outer cuticle. This makes the plant resistant to the rigidity of the environmental climate. This characteristic is responsible for a large amount of anthraquinone, mainly the aloins responsible for the purgative, cytoprotective and anticancer effects of aloe (Bassetti and Sala, 2001; Hamman, 2008; Silva et al., 2010; Yagi and Takeo, 2003).

Effective microorganisms (EMs) include a mixture of live cultures of naturally isolated microorganisms from fertile soils that are used during plant cultivation (Olle and Williams, 2015). The main activity of Effective microorganisms is to increase the soil microfauna, leading to an increase in an increase in field production of fruit and vegetables. Photosynthetic bacteria, present in the EM, synergistically with other microorganisms, improve the absorption of nutrients from the soil and reduce the incidence of disease (Condor et al., 2007). EM technology is based on the inoculation of beneficial micro-organisms into the soil to create a favourable environment for plant growth and health. EMs interact with the soil-plant ecosystem by controlling plant pathogens and disease agents, solubilizing minerals, increasing availability of plant energy, stimulating the photosynthetic system, maintaining the microbiological balance of the soil and fixing biological nitrogen (Olle and Williams, 2015).

Natural Zeolites are a mineral family composed by 54 different species chemically defined as “hydrated allumino-silicates of alkaline and alkaline earth elements” and structurally belonging to the tectosilicates (Passaglia and Sheppard, 2001). Due to their crystal chemistry, zeolites show physical-chemical peculiarities such as high and selective cation exchange capacity (CEC), reversible dehydration, selective molecular absorption, and catalytic behaviour (Armbruster and Gunter, 2001). Therefore, rocks containing more than 50% of zeolites (zeolitites) are widely and profitably utilized in the purification of municipal, zootechnical and industrial wastewaters, as additive in animal nutrition, agriculture and floriculture (Gottardi and Galli, 1985; Galli and Passaglia, 2011).

Because of both the presence of the zeolites and texture of the rocks, zeolitites exhibit high (130-200 meq/100g) and selective (mainly for NH_4^+ and K^+) cation exchange capacity, reversible dehydration, permeability, and high water retention, which are all useful in agricultural, horticultural and floricultural

applications. Accordingly, the zeolitites, itemized by the predominant zeolitic species (chabazitic-zeolites, clinoptilolitic-zeolites, etc), have been recently included in the “ammendanti” (Legislative decree, March 3, 2015). The zeolitites were used in this experiment because they exhibit several interesting features for use in agriculture, horticulture and in particular in tomato (Passaglia et al., 1997), celery (Bazzocchi et al., 1996), courgette and melon (Passaglia et al., 2005), and vegetables and fruit (Passaglia and Poppi, 2005).

The aim of the experiment was to improve the quality and resistance to biotic and abiotic stress on plants of *Aloe barbadensis* Miller and *Aloe Arborescens* Miller by adding chabazitic-zeolites and effective microorganisms to the growing substrates.

MATERIALS AND METHODS

Greenhouse experiment and growing conditions

Trials were conducted in a commercial glasshouse located in Rosignano Solvay, Tuscany, Italy (lat. 43°23' N, long. 10°26' E), under typical Mediterranean climate conditions of coastal areas, in early March 2017. The experiment involved rooted cuttings of 10 cm of *Aloe barbadensis* Miller and 12 cm for *Aloe arborescens* Miller placed in pots ø18 cm, in three different mixtures of substrates to assess their growth and content in metabolites. 30 plants were used for 3 replicas, for 3 theses, 270 plants for each of the two species of Aloe.

The 3 experimental theses in cultivation were:

- **Control (CTRL):** soil for acidophilic 40%, volcanic lapillus 30%, quartz sand 30%, (root wetting every 20 days);
- **Treated (T1):** soil for acidophilic 40%, chabazitic-zeolites 20%, quartz sand 40%, (root wetting every 20 days);
- **Treated (T2):** soil for acidophilic 40%, chabazitic-zeolites 20%, quartz sand 40%, microorganisms EM, (mixture of live cultures of naturally isolated microorganisms, produced by the company Emiko, in Germany) dilution 1:100 (root wetting every 20 days).

All plants were fed with the same amount of nutrients supplied through controlled release fertilizer (5 kg m⁻³ of Osmocote Pro® 3 - 4 months containing 190 g kg⁻¹ N, 39 g kg⁻¹ P, 83 g kg⁻¹ K)

Table 1. Effect of Chabazitic-zeolites and Effective microorganisms on the growth of *Aloe barbadensis* Miller

Treatment	Number of leaves per plant (n°)	Number of plantlets per plant (n°)	Fresh leaf weight (g)	Fresh gel weight (g)	Fresh weight of roots (g)
CTRL	21.92 c	2.86 c	402.55 c	220.22 c	452.26 c
T1	23.50 b	3.41 b	438.15 b	277.44 b	491.81 b
T2	25.52 a	4.47 a	514.30 a	308.22 a	564.43 a

Each value reported in the graph is the mean of three replicates \pm standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test ($P = 0.05$).

blended with the growing medium before transplant. The analysis of zeolites used in the tests determined by X-rays using the Rietveld-Nir methodology (Gualtieri, 2000) had a zeolitic content of: $67 \pm 3\%$ (64% chabazitic-zeolites, 3% phillipsite).

The cation exchange capacity (CSC) determined by exchange with 1 N solution of NH_4^+ according to the methodology described in Gualtieri and Passaglia (2006), showed the following analysis : 210 ± 10 meq/100g (131 meq/100g Ca, 68 meq/100g K, 7 meq/100g Na and 4 meq/100g Mg).

Plant growth and Aloe gel analysis

The experiment lasted 270 days until plants development reached standard level for commercial purposes. At the end of the experiment all plants were subjected to destructive analysis for the determination of: number of leaves per plant, number of shoots per plant, fresh leaf weight, fresh gel weight, fresh root weight. Once every fifteen days the number of new leaves and shoots per plant was counted, 15 plants per treatment, 5 plants per 3 replicas.

In addition, only in *Aloe barbadensis* the content of sugars (Sturm et al. method, 2003), aloin (Waller et al., 2004) and proline (Bates et al., 1973), has been evaluated. 3 leaves per plant, 3 plants per treatment for the evaluation of sugars, proline and aloin have been selected.

Statistics

The experiment was carried out in a randomized complete block design. Collected data were

analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs CoStat (version 6.451) and Excel.

RESULTS

In the experiments, the use of chabazitic-zeolites and EM (Effective Microorganisms) has led to a significant increase in the vegetative and radical development of the plants of *Aloe barbadensis* Miller and *A. arborescens* Miller. In (Tables 1-2), it can be seen that the chabazitic-zeolites (T1) and chabazitic-zeolites +EM (T2) mixtures have led to a significant increase in the number of leaves per plant (Figure 1), in the number of shoots per plant, in the fresh weight of the leaves, in the fresh weight of the gel and in the fresh root weight compared to the fertilized control.

In particular, it is evident that the association of chabazitic-zeolites plus microorganisms has increased the development of plants, compared to the use of zeolites alone. This fact is probably due to the ability of the bacteria to solubilize (by naturally acidifying the substrate) what the chabasite captures during fertigation and then pass it to the root system. Mechanism that in a substrate not colonized by microorganisms is usually slower. In Table 3 also shows how the treatment with chabazitic-zeolites and EM microorganisms can induce and stimulate in the plants of *A. barbadensis* Miller, the accumulation of sugars (fructose and glucose), proline and aloin compared to the fertilized

Table 2. Effect of chabazitic-zeolites and effective microorganisms on the growth of *Aloe arborescens* Miller.

Treatment	Number of leaves per plant (n°)	Number of plantlets per plant (n°)	Fresh leaf weight (g)	Fresh gel weight (g)	Fresh weight of roots (g)
CTRL	23.37 c	3.42 c	468.06 c	247.67 c	502.55 b
T1	24.18 b	3.95 b	499.89 b	293.38 b	591.81 a
T2	26.46 a	5.11 a	575.55 a	369.12 a	626.40 a

Each value reported in the graph is the mean of three replicates \pm standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test ($P = 0.05$).

**Figure 1.** Effect of the substrate with the addition of chabazitic- zeolites and effective microorganisms compared to the control, on the development of the leaves and plantlets of *A. barbadensis* Miller.

Control.

Particular interest is also the increase in the content of aloin in the treated plants (T1 and T2), an anthraquinone with countless activities, not least the laxative, draining and purifying activity that is used a lot by the pharmaceutical and cosmetics industries.

DISCUSSION

The use of zeolites and EM microorganisms can

therefore guarantee, as demonstrated by this evidence, a clear qualitative improvement of Aloe plants in cultivation, in terms of plant growth, root development and production of metabolites useful for nutraceuticals. The main objective of potted plants is the use of substrates and biostimulant that can reduce the use of peat and increase plant quality. As the price of these materials has been rising in recent years as a result of rising energy costs that are reflected in the entire process of production, preparation and transport to farmers. The alternative

Table 3. Influence of chabazitic-zeolites and effective microorganisms on sugars, proline and aloin on plants of *Aloe barbadensis* Miller.

Treatment	Fructose (mg (g DW) ⁻¹)	Glucose (mg (g DW) ⁻¹)	Proline (mg (g DW) ⁻¹)	Aloin (mg (g DW) ⁻¹)
CTRL	80.95 c	30.26 b	0.75 c	152.89 c
T1	91.18 b	32.48 ab	0.87 b	164.37 b
T2	93.52 a	34.40 a	1.17 a	172.95 a

Each value reported in the graph is the mean of three replicates \pm standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test ($P = 0.05$).

materials used often create problems for plants related to rooting or water and salt stress. Zeolites commonly utilized in agriculture for the cultivation of horticultural and ornamental crops (Passaglia et al., 1997; Bazzocchi et al., 1996; Passaglia et al., 2005; Prisa and Burchi, 2015; Prisa, 2016; 2017a,b) and for the reduction of NH_4^+ content in the liquid manure in the pig farms (Bergero and Passaglia, 1994; Passaglia and Marchi, 2001), could resolve in part this problem. These minerals, added to peat or to other organic compost at 20% content, are practical to use, easy to mix to the soil or to other substrates, also for soilless cultivation. The active nutrients and water content result always available to plant and the adsorbed fertilizing elements are safe from the risk of run-off due to rain or irrigation (Passaglia and Prisa, 2018).

Also Effective Microorganisms can increase plant quality, in particular in tomato and pumpkin plants (Olle and Williams, 2015). EM microorganisms also lead to an increase in calcium content by reducing the incidence of insect disease and improves the quality and preservation of fruit and vegetables (Pavlovic et al., 1998). Some scientists have shown that EM can increase fruit weight, yield, photosynthesis (Idris et al., 2008). EM applied with green manure significantly increased tomato yields and in the third year were comparable to those obtained with chemical fertilizers (Marambe and Sangakkara, 1996).

The results of this research have shown that chabazitic-zeolites and Effective microorganisms can improve some traits of plant quality in *Aloe barbadensis* and *A. arborescens*, such as number of leaves per plant, number of shoots per plant, fresh leaf weight, fresh gel weight, fresh root weight. In particular, the use of chabazitic-zeolites and

effective microorganisms has led to an increase in the content of sugars (glucose and fructose) and proline and aloin in *Aloe barbadensis*. This could be associated with a higher water and mineral content, influenced by zeolite (Prisa and Burchi, 2015; Prisa, 2016; 2017a,b), and with a higher root development, affected by the action of micro-organisms (Olle and Williams, 2015).

CONCLUSION

These trials showed several benefits that can be obtained through the use of chabazitic-zeolites and effective microorganisms: improvement of quality in *Aloe barbadensis* Miller and *A. arborescens* Miller, in terms of number of leaves and shoots per plant, fresh weight of the leaves and roots, fresh weight of the gel. In *A. barbadensis* also increase the content of sugar and nutraceutical metabolites.

Chabazitic zeolites and effective microorganisms as demonstrated in other experiments (Passaglia and Prisa, 2018), also prove to be a viable alternative to conventional techniques, to improve the use of fertilizers and irrigation water in potted plants

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